Technical Bulletin No. 67 May, 1925

Investigations on The Blackleg Disease of Potato

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For years, serious losses from a potato tuber rot, both in the field and in storage, have been reported from various sections of Michigan. As commonly reported, the affected tubers became blackened, and decayed with a soft, slimy, ill-smelling rot. Reports on specimens sent to various plant pathological laboratories, showed that a considerable difference of opinion existed as to the causal organism; and it was to clear up this matter, as well as to make a more thorough study of the disease, with especial reference to its host relations and method of dissemination as a basis for control measures, that the present investigations were undertaken in 1919. While much of the preliminary work was done during that and the following year, the investigations have been continued until the present time. The disease which caused the rot of the tubers was found to be blackleg caused by *Bacillus atrosepticus* van Hall.* The results obtained while confirming work of other investigators, in part, have opened up some new points of view with reference to this disease.

SIGNS OF THE DISEASE

The disease in Michigan presents a picture similar to the blackleg disease of potato as described by Morse[1]** in Maine, Harrison[2] in Canada, Pethybridge and Murphy[3] in Ireland, van Hall[4] in Holland, and Appel[5] in Germany. As the name indicates, the disease is characterized by an inky blackening of the stem usually at the ground region. This condition is not, however, the first indication of the disease, but on the other hand, is the characteristic development which confirms the diagnosis. The first signs are the gradual fading of the deep green color of the foliage into yellow-green; later, the plants take on a distinct yellow color. This sickly appearance is accompanied usually by an upturning of the leaflets and a tendency of the branches to grow upward forming a more or less compact top. The plant gradually dies as the base of the stem is rotted away by the bacteria which cause the disease. The diagnosis of the disease in the field is readily confirmed by pulling the affected plants. The stems, already considerably rotted, offer no resistance to being pulled up and have an inky, black appearance. (PLATE I.) Seasons of abnormal rainfall present conditions which are

*The name, *Bacillus atrosepticus* van Hall, is used in this paper to designate the causal organism of blackleg. The specific names *phytophthorus* (Appel), *solanisaprus* (Harrison) and *melanogenes* (Pethybridge and Murphy) are also used by various authors to designate the same organism.

**The numbers following the names of authors mentioned refer to the papers listed in the bibliography at the close of this paper.

very favorable to rapid bacterial growth and during these periods, blackleg presents some different aspects. The disease progresses rapidly and involves more of the top than in dry seasons. Succulent stems are rotted in a very short time and the plants fall over before the leaves have had time to assume a light green or yellow color. In these cases, the stems may show a water-soaked appearance eight or ten inches above the surface of the ground. Instances are known in which the blackening extended to the tips of the branches. The blackleg germ makes rapid progress in the pith area of the stem and this in many cases results in hollow stalks. Under less favorable conditions, when the disease progresses very slowly aerial tubers may be formed as in the case of the Rhizoctonia disease of potatoes.

Occasionally diseased plants are found, especially during the latter part of the growing season, which differ somewhat from the typical symptoms of blackleg as described above. These plants show a blackening of the succulent portions of the stems above ground while no external discoloration appears on the basal or underground portions of the stems. In regard to similar cases, Morse[1] states: "In all the cases observed, however, a closer examination has shown that the pith has been destroyed between the base of the stem and the externally diseased area above ground." Specimens of this type of blackleg sent to the Michigan station plant pathological laboratory, in 1924, showed discoloration of the vascular system which extended from the base of the stems to the above ground portions. The pith of these stems, although slightly discolored, was not rotted. Isolations made from the discolored vascular system near the base of the stems yielded the blackleg organism, thus confirming the work of Ramsey as reported by Morse.[1] The difference in symptoms is due probably to slow and retarded growth in the underground portions of the stem, which because of its woody texture and relative dryness is able to limit the invasion of the bacteria, thus causing the latter to be confined to the vascular system and the pith. Upon reaching the more succulent top-growth, however, the bacteria are able to break through and cause a rotting and blackening of the outside of the stems.

The disease in the tubers, as seen in the field, is characterized by a rotting which proceeds from the stem end, the infection having spread from the diseased seed piece to the plant stem and from the latter to the stolon and finally to the new tuber. The writers have observed but one case of lateral infection of a tuber while in the soil and this was brought about by the immediate contact of the tuber with a diseased stem. The skin of the tubers which are rotting in the soil, presents a gun-metal appearance over the decaying portions. Under moist conditions, young tubers are rotted very quickly, whereas if dry conditions follow, the rotted portions dry up and may give the tubers a truncated appearance. In this condition, the advance of the bacteria is checked until more favorable conditions allow further rotting to progress. Early stages of tuber invasion and those in which the bacterial activity has been arrested, show, upon slicing the stem end of tubers, a ring discoloration somewhat like typical Fusarium invasion. Tubers in the soft rot stage produced by the blackleg organism when cut open, show a white, creamy bacterial growth upon the flesh of the tuber, the sound portion always showing a black line or border. There is also a tendency toward canal formation. (Plate II.)

PREVIOUS HISTORY

Owing to the wide distribution of blackleg, the disease occurring in practically every country where the potato is grown at all extensively, and its general importance, considerable literature on the disease has accumulated. For extensive reviews of the early literature,—Morse,[1] Pettybridge and Murphy,[3] van Hall[4] and Appel[5] should be consulted, as only the more recent contributions are included in the following review.

Morse[1] (1917) in summarizing his nine years' work on the blackleg disease in Maine gave a review of the literature, the geographical distribution as well as economic aspects of the disease, control measures and a comparative study of the associated organisms from many localities. He reported, as a result of his observations on the sources of infection and wintering over of the organism, that diseased tubers and not the soil were the spring source of infection.

Rosenbaum and Ramsey[6] (1918) investigating the influence of temperature and precipitation upon the blackleg disease of potato, found that the severity of the disease was closely correlated with temperature and precipitation and was dependent upon them. These investigators conducted experiments on wintering over of the disease both in Maine and Virginia and confirmed the statements of Morse.

Ramsey[7] (1919) reported studies on the viability of the potato blackleg organism after being subjected to various climatic and environmental conditions. These experiments supplemented in a minor way the more extensive experiments of Rosenbaum and Ramsey. In this work infected tubers were subjected to low temperatures and these invariably proved fatal to the blackleg bacteria. He obtained negative results from attempts to inoculate plants by watering them with infusions containing the bacteria.

Artschwager[8] (1920) in a study of the pathological anatomy of potato plants affected with blackleg, found that such plants show an increase in strongly lignified vascular tissue and a transformation of part or most of the parenchyma cells of the cortex and pith into sclereids. He noted also the occurrence of protein crystals in the cells of the leaves, and states that under normal conditions protein crystals have been observed only in the peripheral cell layers of the cortex of the potato tubers.

Murphy[9] (1921) substantiated by field experiments the views of other investigators, that the disease does not spread in the field and does not attack a plant unless the set was already infected. He states: "The presumption is that blackleg is carried only by the tubers because all available evidence shows that the infection does not spread in the soil to any appreciable extent, if at all, and does not in any case survive the winter even in the mild climates of Ireland or Virginia."

Jennison[10] (1921) described cultural work on 12 strains of the blackleg organism including several of Morse's strains and concluded that they were all alike. After reviewing the various names applied to the causal organism, the name *Bacillus atrosepticus van Hall* was chosen as the correct name to be used.

Shapovalov and Edson[11] (1921) demonstrated that a bacterial, field decay of potato tubers occurring in Colorado potatoes grown under irrigation, which had not been explained adequately was caused by *Bacillus phytophthorus*. The disease, however, they pointed out took on a form atypical of

the familiar manifestation of blackleg in the east, and on account of this difference had been assumed in some instances to be due to "sunscald" or freezing injury. In other instances, it was thought to have been confused with "jelly-end rot" caused by *Fusarium radicicola Wollenw.* or by *F. oxysporum* Schlecht., and with "leak" (*Pythium debaryanum* Hesse.)

MICHIGAN OBSERVATIONS AND EXPERIMENTS

As noted in the above review of the more recent literature, Shapovalov and Edson[11] reported differences in the symptoms of blackleg manifested in potatoes grown under irrigation and under conditions as existing in Maine. Since it was thought at one time by certain observers familiar with blackleg in Maine, that the disease in Michigan did not seem typical of blackleg but rather resembled a Fusarium disease which had been observed in Minnesota, preliminary investigations were undertaken in 1919 to determine the nature of the Michigan disease.

Diseased material of the type in question was obtained from two widely separated fields in Alger county, Michigan, and dilution plates were poured. From these plates nine bacterial colonies were isolated, five of which, when inoculated into raw tubers and into healthy, growing plants produced characteristic symptoms of the disease. In no case were any of the molds which developed upon the plates found to be Fusaria. This work showed the disease in question was of bacterial origin. Since then, these isolations and others which have been obtained from diseased plants from a great many different Michigan farms, have been tested out in comparison with authentic strains of *Bacillus atrosepticus* obtained through the courtesy of the Wisconsin and the Maine Experiment Stations and all have produced similar blackleg effects. There can be no question as to the etiology of the blackleg disease of potato in Michigan, and certainly a Fusarium is not concerned. The organism is a bacillus behaving in culture and pathogenicity identically like authentic cultures of *Bacillus atrosepticus.*

ECONOMIC LOSSES

The losses resulting from blackleg vary with the seasons. During seasons which are cool and wet, conditions are very favorable for disease production and much greater losses follow than is the case during warm seasons of little rainfall. As to the importance of the disease in this and different countries, the reports of various investigators may be summarized as follows:

Germany:

Frank[13] and Appel[5]: The disease is common, widespread and often destructive.

France:

Delacroix[14]: Perhaps wider spread than this investigator supposed because of the confusion between this disease and "brunissure."

Ireland:

Pethybridge[3]: Ninety-four per cent of diseased plants produced by selecting sound seed tubers from an infected crop of the previous season.

Canada:

Harrison[2]: Estimates total loss through rot 10-75 per cent of the crop in Province of Ontario, 1905.

Murphy[9]: In parts of the maritime provinces of Canada 10 per cent of total crop in 1915.

United States:

Morse[1]: Extensive observations in Aroostook county, Maine, covering a period of five years revealed in many fields only scattered blackleg plants and not infrequently these amount to 1 or 2 per cent, 5 per cent of diseased plants being considered by the growers as representing a severe attack. Losses of 10 or 15 per cent or more are by no means unknown, however.

Coons[15]: Reports that losses of 25 to 75 per cent were not uncommon in the Upper Peninsula of Michigan in 1915.

Weston:* In a letter presents the following information on the occurrence in Michigan of blackleg in stocks of certified seed potatoes and table stock: "There is no doubt but that blackleg is more prevalent in non-certified fields by several per cent than will be found in certified fields in the northern part of the state. The control of blackleg is one of the more serious problems found in connection with the certifying of seed potatoes."

From the reports of Coons and Weston noted above, it is seen that potato blackleg is a serious disease in Michigan and that its elimination is a problem which confronts the grower of certified seed potatoes as well as the grower of table stock. The persistence of the disease in certain stocks of certified seed emphasizes the importance of strict adherence to the recommended control measures.

HOST RELATIONS

Resistance and susceptibility of varieties:

Practically all of the investigators who have studied the blackleg problem have noted the differences in resistance and susceptibility of varieties of potatoes. Appel[5] was the first to note this difference, and Harrison[2] also found that some varieties seemed to be more susceptible than others. The latter investigator, however, in variety tests found out that many varieties which showed resistance in the field were comparatively susceptible when subjected to artificial inoculation. Morse[1] in studying the disease in Maine observed

*J. W. Weston is Extension Potato Specialist of the Michigan Agricultural College and his field studies in the potato growing sections of the state have made him intimately acquainted with the blackleg situation. Mr. Weston's reports very early called attention to the seriousness of this problem and led to the inception of this study.

that the Irish Cobbler, an early maturing variety, was more susceptible to blackleg than the Green Mountain, which is a late maturing variety, and goes on to state that: "As long as Maine potato growers planted this variety exclusively, blackleg was of minor consequence."

Field observations in Michigan, supplemented by laboratory and greenhouse tests have led to the conclusion that the Green Mountain is not so resistant as one would be led to believe from the observations of Morse. Laboratory tests on tubers of eight varieties were conducted and from the data obtained differences in resistance and susceptibility were judged. These data showed that the Bliss Triumph, a very early maturing variety, was more resistant to blackleg than was the Green Mountain, which proved to be only slightly more resistant than the Russet Rural, another late variety.

To determine whether resistance and susceptibility as expressed by tuber inoculations was correlated with similar resistance and susceptibility of stems, healthy succulent plants of the same eight varieties were inoculated with approximately equal amounts of a virulent 24-hour broth culture of *B. atrosepticus,* the inoculations being made on the stems just above the surface of the soil. The plants were of the same age and were grown in the greenhouse. After inoculation, the plants were placed in a Wardian chamber where a very humid condition was maintained. Ten days after inoculation the plants were examined and, as in the case of tuber inoculations, differences in resistance and susceptibility were apparent. The Green Mountain and Bliss Triumph varieties, however, instead of being resistant were the first varieties to succumb to the attack of the disease, while the Irish Cobbler and Rural New Yorker types proved to be more resistant.

A comparison of the resistant and susceptibility of varieties as expressed by tuber and stem inoculations may be made by referring to the following table:

TABLE I.—Relative resistance and susceptibility of potato varieties to blackleg: Varieties arranged in the order of resistance shown.

TUBERS	GROWING PLANTS
Resistant:	*Resistant:*
1. Bliss Triumph	1. Sir Walter Raleigh
2. Green Mountain	2. Carmen No. 3.
3. Russet Rural	3. Irish Cobbler
4. Irish Cobbler	4. Russet Rural
5. Early Ohio	5. Early Ohio
6. Early Rose	6. Early Rose
7. Sir Walter Raleigh	7. Bliss Triumph
8. Carmen No. 3.	8. Green Mountain
Susceptible	*Susceptible*

The results here presented make it clear that statements as to resistance or susceptibility of varieties need to take into account the part of the plant attacked, since varieties with resistant stems may have very susceptible tubers and vice versa. It would seem that recommendations for field practice as to probable resistance of varieties to the blackleg disease cannot be drawn from tuber or stem inoculations alone. Extensive trials in the field and greenhouse as well as in the laboratory must be made before definite conclusions as to varietal resistance can be drawn. It seems unlikely if any of the commonly grown varieties will prove resistant enough under all conditions to prevent blackleg losses entirely.

Other hosts for the parasite:

Appel[5] was the first to attempt to ascertain whether the organism which was responsible for blackleg in Germany was parasitic on other hosts. This investigator succeeded in infecting cucumber, Windsor bean, carrot, Teltower beet, lupine and tomato with *B. phytophthorus,* but was not successful in infecting sugar beets, mangels, Pelargonium, and the grains.

Harrison[2] reported successful results from inoculations on other hosts with *B. Solanisaprus.* Positive results were obtained from inoculating tomato, pepper, (Capsicum annum), and cucumber, while negative results were obtained from inoculations into *Solanum melongena, Physalis sp., Datura stramonium, Schizanthus pinnatus, Collinsia verna, Salpiglossis sinuata,* and *Ipomoea purpurea.*

In similar experiments the writers have obtained positive results from inoculations into tomato and *Nicotiana rustica.* Negative results have been obtained from frequent inoculations into Petunia, (*Solanum dulcamara, Datura stramonium, Physalis spp., Nicotiana tabacum,* and a solanaceous plant sold by seedsmen under the name of Wonderberry (*Solanum nigrum*).

From the above experiments it is evident that the causal organism of the blackleg disease of potato is parasitic on a number of other hosts besides potato. The disease has not been observed on these other susceptible hosts in nature.

Amount of inoculum:

It has been noted by Smith[12] "—that in culture media not exactly adapted to the needs of an organism, a scanty inoculation may not give any growth—not even after a long time—whereas a copious one will lead to a growth which gradually clouds the fluid or covers the solid.—The only explanation I can think of is that a multitude of bacteria is stronger than a few, and thus by union are able to overcome obstacles too great for the few. The same fact comes repeatedly to the attention of the animal pathologist as a result of his inoculations. The animal body, we must assume, is often able to overcome and destroy a few hostile organisms, where it would not be able to defend itself against many; otherwise whole races would be exterminated by natural infections. The same is undoubtedly true of plants. The *modus operandi* in plants is not altogether clear. We may advance several hypotheses: (1) The formation of a resistant cork-layer before the bacteria have multiplied to such an extent as to prevent cell-division; (2) the destructive action of antiseptic plant substances, e. g., acids, before these can be neutralized or destroyed by the substances produced by the multiplying bacteria. In some instances, the introduction of a very considerable mass of bacteria seems to be necessary to induce disease; in other cases a very few are sufficient. It would be extremely interesting to know the minimum number capable in any given case of inducing disease."

In order to determine the effect of amount of inoculum in the case of the blackleg disease of potato, the following experiment was performed. Dilutions of 1:100, 1:10,000, 1:100,000, 1:1,000,000 were made from a virulent 72-hour broth culture of *B. atrosepticus.* Bliss Triumph tubers were disinfected with corrosive sublimate and cut into small pieces by means of a sterile knife. These slices were then placed in small preparation dishes upon pieces of absorbent cotton saturated with distilled water. By means of a sterile

pipette one drop of the broth culture, undiluted, was placed on each of ten tuber slices. The various dilutions were treated in like manner using a different sterile pipette of the same size for each dilution. One drop of each dilution was placed also in tubes of melted nutrient agar and plates poured at once. Data obtained from this experiment are recorded in the following table:

TABLE II.—Effect of amount of inoculum.

Dilution	No. Colonies on Plates	Incubation Period	Growth on Individual Slices									
			1	2	3	4	5	6	7	8	9	10
Undiluted culture	Very numerous	2 da.	+	+	+	+	+	+	+	+	+	+
1:100	19,650	2 da.	+	+	+	+	+	+	+	+	+	+
1:10,000	556	3* da.	+	−	+	+	+	+	+	+	+	+
1:100,000	96	5* da.	−	+	−	−	+	+	+	+	+	−
1:1,000,000	8	5* da.	−	+	−	+	−	−	−	+	−	−

*No rotting occurred before this period.

An examination of the above data reveals several interesting relations. It is apparent that a relationship exists between the amount of inoculum and the length of time required for the appearance of rotting. While growth appeared after the usual incubation period of two days in the cases of the undiluted broth culture and 1:100 dilution, it was not evident until three days in the case of the 1:10,000 dilution, and not until five days in the highest dilutions. That the growth in these two dilutions, caused by approximately 96 and 8 organisms respectively, was considerably weaker than in the others, also is significant. The 96 (approximately) organisms, per drop, out of a possible ten inoculations, produced six which were positive while the 8 (approximately) organisms, per drop, were able to produce rotting with a much smaller number of slices, which shows that with these numbers we are approaching the smallest numbers which can successfully attack tuber slices to produce rotting and here the process is slow and erratic.

The effect of various quantities of inoculum on slices of raw tubers was tested out in another manner. Raw-tuber slices of Bliss Triumph variety were inoculated by placing varying quantities of a virulent 3-day-old broth culture on the cut surfaces by means of a 3 mm. platinum wire loop. The tuber slices were not pricked with a needle as is the method ordinarily employed in making inoculations. After inoculation the slices were placed in moist chambers, and 48 hours later showed marked differences. Those slices which were inoculated with very thin films of broth culture, instead of showing creamy bacterial growth as in the cases of inoculations with one or more loopfuls of the culture, showed instead an overgrowth of host tissue, as if from stimulation of cellular activity, which was exemplified by a wart-like protuberance, or an oedema formation. (Plate III.) It was at first

thought that this phenomenon might be due to rapid division of cells, the minute quantity of inoculum being the exciting factor. Microscopic examination, however, revealed the fact that these protuberances instead of being composed of great numbers of minute cells were made up of giant cells, and that these cells contained very few starch grains as compared to the number found in normal cells. As a probable explanation of the phenomenon it is suggested that as a result of the action of the organism or its products upon the cells, the cell-walls become less rigid, and the starch grains are changed into sugar. The resulting increased osmotic pressure acting on the more elastic cell membranes leads to the increased size of cells.

These experiments confirm the belief of Smith[12] that a multitude of bacteria is stronger than a few, and show that potato tubers are able to resist the attack of minute quantities of blackleg bacteria, while larger amounts are able to break down this resistance and cause the rotting of the tubers.

Enzyme studies:

Harrison[2] was able to produce typical blackleg lesions by the use of the chemical products (enzymes) of the growth of *Bacillus solanisaprus,* and states that the solvent action of the enzyme on the cementing substance of the cells was quite marked. The writers were able to cause the softening of raw-tuber slices by placing small quantities of a dry alcoholic precipitate, by means of a sterile scalpel and platinum needle, upon the freshly cut surfaces. This enzymic extract was made by mashing 12 tuber slices, severely rotted by blackleg, in 50 cc. of water, the mixture then being filtered through filter paper and precipitated with 90 per cent alcohol. The precipitate was dried on glass plates.

In order to ascertain whether the enzyme produced by the blackleg organism is similar to that produced by *Bacillus carotovorus,* as described by Jones[16], and to compare the action of the bacteria themselves with enzymic action, the following experiment was performed: Upon six potato and six carrot slices a small quantity of the dry alcoholic precipitate, prepared as described in the preceding paragraph, was placed while potato and carrot slices were inoculated with one drop of 24-hour broth cultures of *B. atrosepticus,* and Spieckerman's bacillus. The following results were obtained:

TABLE III.—Effect of enzymic extract on raw potato and carrot: Comparison of alcoholic precipitate from rotted potatoes with pure cultures of bacteria.

		On Potato			On Carrot			
	Inoculum	No. Inocs.	24 hours	48 hours	No. Inocs.	24 hours	48 hours	72 hours
A	Alcoholic precipitate	6	Softening	Marked Softening	6	Slight Softening	Slight Softening	Marked Softening
B	*Bacillus atrosepticus*	1	—	Slight creamy growth	1	—	Slight discoloration	Slight discoloration
C	*Spieckerman's bacillus*	1	—	—	1	—	Slight discoloration	Slight creamy growth
D	Distilled water	1	—	—	1	—	—	—

Microscopic examination of the softened tuber and carrot slices, subjected to the alcoholic precipitate revealed many free cells, showing that the enzyme

acts on pectin of the middle lamella, which would suggest that the enzyme is pectinase. The fact that carrots were softened also, though not so rapidly as were potatoes, indicates that the enzyme produced by *B. atrosepticus,* if not identical, is very closely related to that produced by *B. carotovorus.*

The data recorded in Table III also show that the enzymic activity is much more vigorous in dried extract than in bacterial growths. Whereas in 24 hours in the former there was evidence of dissolution of the plant tissue, in the latter there was none. There are several other points of difference between enzymic and bacterial action. The potato slices softened by the former are not blackened as is the case when tubers are rotted by the blackleg germ, and the enzymic dissolution of the potato slices and carrots is not accompanied by the disagreeable odor which accompanies the rotting of tuber slices when inoculated with the live organism. On the contrary the odor is rather fragrant and especially so in the case of the enzymic softening of carrot slices.

The Toxic Substance produced by B. atrosepticus:

It was observed from the above enzyme experiments that there was no discoloration of the tubers as a result of the action of the enzyme, and as blackening both in tubers and on the stems of infected plants is such a constant characteristic of blackleg, the question was raised as to what caused the discoloration. As the enzyme was unable to produce discoloration, it was thought very probable that the blackleg organism must in addition produce a toxic substance which brings about the blackening. This theory was confirmed readily by placing a virulent 24-hour broth culture of *B. atrosepticus* within a thin sterile collodion sack about 8 mm. in diameter and 4 cm. long, and then placing the sack on a sterile raw potato plug in a sterile test tube. The bacteria and substances of colloidal nature, the enzyme, etc., which are produced in cultures were kept inside of the sack while those substances having smaller molecular dimensions (e. g., the toxic substances) were able to diffuse through to the surface of the potato plug. This diffusion was accompanied by a brownish discoloration resembling typical blackleg staining in its first stages of development.

The presence of a toxic substance was demonstrated also by placing leaves from healthy rapidly growing potato plants in test tubes containing beef and potato broth cultures of *B. atrosepticus* using the collodion sack method to keep the germs from direct contact with the leaf petioles. In this test the part of the leaf petioles which were kept submerged in the liquids was enveloped in a thin collodion sack. In three days these leaves were wilted while the check, which was enveloped in a similar sack and kept in distilled water, was still turgid. (Plate IV.)

From the above studies of the relations of the host and parasite, it seems extremely likely that two agencies operate in the mechanism of the attack of the blackleg organism upon potato. *B. atrosepticus* produces a pectin-dissolving enzyme, which works upon potato as well as upon carrot tissue and a toxic substance which brings about discoloration of potato, and the two working in conjunction, under favorable conditions produce the discoloration and rotting characteristic of blackleg. Viewed in the light of these two factors, the rotting effects of heavy dosage and the oedema formation with light dosage, seem explainable on the basis of relative amounts of toxic substance present or produced. The close relationship of *B. carotovorus* and

B. atrosepticus has been noted by many observers. *B. atrosepticus* seems to possess the power of producing a toxic substance which affects potato cells so as to allow blackening, probably oxidation, to ensue, while *B. carotovorus* which softens potato tissues under some conditions without blackening, does not possess this power.

The difference in pathogenicity between *B. carotovorus* and *B. atrosepticus* is thus found to rest upon differences in toxic substances.

WINTERING AND FIELD DISSEMINATION

The question of dissemination of potato blackleg has received the attention of many investigators including Morse,[1] Rosenbaum and Ramsey,[6] Ramsey,[7] Murphy,[9] and others. From field experiments on the wintering over of the disease in diseased tubers, supplemented by laboratory experiments and numerous field observations, these authors conclude that the bacteria do not winter over in tubers left in the soil and that they do not spread in the soil to any appreciable extent even in as mild a climate as that of Virginia.

It has been conceded generally, by most investigators that diseased tubers kept in storage over winter are the main source of infection. These tubers under favorable conditions rot and cause the rotting of adjoining healthy tubers. The bacterial slime may spread also over the surface and into the eyes, cracks, or bruises of otherwise normal tubers and as a result these tubers become diseased when planted the following year. Although Morse[1] and others have noted that the organism is killed readily by desiccation when it is dried on a smooth glass surface, the writers have found that under other conditions the organism will retain its vitality for a much longer period. For instance, Morse found that the organism when dried on small glass discs at room temperature was dead in less than half an hour, whereas the writers have found that the organism will retain its vitality for four days when dried on a silk thread under similar conditions. (See Table IV.)

TABLE IV.—Desiccation of the blackleg organism, Bacillus atrosepticus.

Medium	Where Kept	Tested on Raw Tubers and Nutrient Broth					
		1 day	2 days	3 days	4 days	5 days	6 days
Cover glass	Desk drawer	−	−	−	−	−	−
Cover glass	Desiccator	−	−	−	−	−	−
Silk Thread	Desiccator		+	+	+	−	−
Silk Thread	Desk drawer	+	+	+	+	−	−

Stevens[17] obtained more striking results in his desiccation experiments with *Pseudomonas citri*. He found that the organism failed to germinate after being exposed two weeks on glass slips to laboratory air, while pieces of cloth wetted with suspensions of the organism and kept under similar

conditions showed a very large number of organisms alive after a drying period of five weeks.

The above results indicate that the length of time an organism can withstand desiccation varies with the medium upon which it is dried, and that under conditions to which blackleg organisms are subjected in nature there may be sufficient moisture or protection from desiccation to permit the organism to live until more favorable conditions arise.

From a study of the longevity of soil micro-organisms Giltner and Langworthy[18] conclude that the survival of non-spore bearing bacteria in air-dry soil is due, in part, to the retention by the soil of moisture in the hygroscopic form, which would indicate that the blackleg organism would be able to persist in soil for long periods were it not for the intervention of other factors to be discussed later.

Overwintering in tubers in soil:

In regard to wintering of the disease in tubers which remain in the soil over winter Morse,[1] basing his opinion upon field observations, makes the following statement: "On fields which are planted with potatoes the second time in succession there is usually quite a percentage of volunteer plants which spring from tubers which remained in the soil over winter. These plants are frequently easily recognized by their irregular occurrence on the sides of the rows or between hills. The writer (Morse) has never seen such plants affected by blackleg. This observation was quite unexpected, for it seems not unreasonable to suppose that, if the tubers were sufficiently protected from frost to be able to germinate, the bacteria causing the disease might live over in the soil in such infected tubers as well as in storage." Ramsey[7] in studying the wintering over of the bacteria, inoculated healthy, disinfected potato tubers with a pure culture of a strain of *B. atrosepticus*. After being left in a moist chamber for 48 hours the tubers showed marked rotting at the point of inoculation and were then packed in cracked ice in a refrigerator. Every 24 hours one of these tubers was taken out and planted. Out of the eleven tubers which were planted only two germinated but the sprouts were killed immediately by blackleg. All other tubers decayed without showing any sprouts above ground. Plates which were poured from the tubers before planting showed that the number of bacteria was reduced to a minimum after 264 consecutive hours. Ramsey offers this as an explanation of the reason why blackleg is never found in volunteer plants in Maine, and states: "Evidently the infected tubers, which remain buried in the fields at digging time, decay to such an extent that they are unable to germinate, or if they do germinate, the sprouts are immediately killed by the organism. Therefore, all volunteer plants which make their appearance in the spring are free from blackleg."

Rosenbaum and Ramsey[6] in more extensive experiments on the wintering over of the blackleg organism in naturally infected and artificially inoculated tubers found that: "In no case was the blackleg organism found to live over a winter in the soil or in the tubers remaining in the soil."

In the writers' opinion the conditions of the above experimentation are not comparable to those which obtain in nature. It is to be noted that markedly rotted tubers were used whereas often under field conditions affected tubers are found which show little or no external indication of blackleg,

and it is not unlikely that the disease could be carried over winter in such slightly invaded tubers. Soil conditions during the winter, or at least those of the Upper Peninsula of Michigan, cannot be compared to conditions represented by Ramsey's[7] cracked ice experiment, because in ordinary years the ground is not frozen before it is covered with snow and permits the wintering of potatoes at a comparatively low but not freezing temperature. One of the writers (Kotila[19]) has observed from experiments on chilling and frost injury, that potatoes which have been exposed to freezing temperatures decay very rapidly through the invasion of secondary organisms, and it is possible also that these secondary bacteria swamped and crowded out the blackleg organisms in Ramsey's experiments.

In order to secure information in regard to the wintering of the blackleg organism under conditions existing in the Upper Peninsula of Michigan, a plot of Green Mountain potatoes, approximately one-quarter of an acre in size, in which blackleg was present was left undug in the fall of 1918. In the spring of 1919 this plot was dug and a portion of the crop was spread out upon a clean floor of the loft of a barn to greensprout. Six bushels of this green-sprouted stock were selected carefully, all tubers showing evidence of rotting being discarded. Two bushels were disinfected with mercuric chloride and two bushels with formaldehyde in the usual manner, while the remaining two bushels were left untreated as a check. The potatoes were planted with a machine planter, the mercuric chloride treated tubers being planted first, followed by the formaldehyde treated and untreated tubers, respectively. To avoid the possibility of soil infection, the tubers were planted in soil only recently brought into cultivation and which had borne no previous root crop. Careful examination was made of these plots twice a week throughout the entire growing season and each diseased hill was staked as found in order to avoid duplication in recording the number of diseased hills in each plot. The results obtained from this experiment are shown in the following table:

TABLE V.—Over-wintering of blackleg in tubers remaining in the soil at the U. P. Exp. Station, Chatham, Michigan, 1918-1919.

Treatment	Number blackleg hills found during season	Per cent
Mercuric chloride, 4 oz. to 30 gal., 30 min.....	2	0.4
Formaldehyde, 1 pint to 30 gal., 15 min.....	7	1.4
Untreated.....	35	7.2

Plates were poured from diseased plants, the causal organism isolated, and its pathogenicity proved by inoculation into raw tubers and into healthy growing plants. Further evidence of blackleg was found in three plots of the same spring-dug seed stock planted in another field. The above experiment and observations indicate that the blackleg disease can winter in tubers which remain in the soil under conditions existing in the Upper Peninsula of Michigan. The relation of treatment to percentage of blackleg which appeared is significant.

Overwintering in the soil:

Having found that under certain conditions, the organism causing potato blackleg can survive the winter in infected tubers, the question was raised as to the possibility of it wintering over directly in the soil. Both Appel[5] and Harrison[2] believed that the organism remained in the soil and in their control measures recommend rotation of crops. Morse,[1] drawing conclusions from observations made of fields planted to potatoes two years in succession which during the second year showed either not any or reduced numbers depending upon, in his opinion, the thoroughness of seed treatment, states: "— infected seed potatoes are the sole source of infection and distribution and that the disease does not live over the winter in the soil." Rosenbaum and Ramsey[6] arrived at the same conclusion after attempting to isolate the organism from soil in various parts of their wintering over test plots. These investigators poured many series of plates and made a great number of isolations, all of which proved to be non-pathogenic when inoculated into raw tuber slices and into growing plants. The difficulty experienced in endeavoring to pick up colonies of the blackleg organism from plates poured from soil samples is, of course, obvious and militates against the force of their conclusions. There is such a great number of soil organisms, which develop upon agar plates, very similar to the blackleg organism in appearance, that the chances of picking up the right organism are very small.

The following experiment is noteworthy in further confirmation of the positive statements of other workers: In this experiment three kinds of soils were used, clay, sand and muck. Each of these was thoroughly mixed after receiving two liters of a decoction made up of severely rotted tubers and water, this quantity being sufficient to saturate the soils. Sufficient soil of each kind was used to fill two large wooden pails. One series was kept in the greenhouse while the other was allowed to winter over outdoors. In the spring each soil was thoroughly mixed again and an average sample taken according to methods usually employed in soil analysis work. Then, instead of attempting the almost futile task of isolating the organism from the soil by the plating method, raw tuber slices were inoculated with small portions of the soil, thus allowing the tuber slices, the natural host, to do the selecting. One hundred and eight tuber slices were inoculated with small quantities (approximately one-half gram) of these soils, eighteen slices being used for each of the six samples taken. After three days no rotting was observed so a thin layer was cut off of each slice and the fresh surface reinoculated with a small quantity of soil as before. After five days, rotting was evident on few slices and after a still longer incubation period it was observed that 90 of the possible 108 tuber slices were rotting. It should be recorded that from the soils which were used as inoculum in this experiment, there were produced on the potato slices mixed cultures of bacteria and fungi, and that some of the slices showed discoloration not entirely dissimilar to the blackening which appears on tubers from blackleg. It is to be noted that a long time period elapsed before rotting began, many times greater than the regular period for blackleg bacteria to produce rot. Certain soil fungi, and associated bacteria, are capable of producing discoloration and rotting of tubers and these are likely to have been responsible for the rotting in this experiment. Attempts to isolate the blackleg organism directly from the rotting slices or from plants inoculated from discolored tuber slices resulted in failure. It is doubtful, therefore, if any of the rotting was of the *B. atroscpticus*

type. The conclusion, therefore, seems justified that under the conditions of this experiment the blackleg organism did not over-winter in the soil, either in the greenhouse or out-of-doors.

Further confirmatory evidence that the blackleg organism does not survive Michigan winters directly in the soil was obtained from field experiments conducted at the Upper Peninsula Experiment Station, Chatham, Michigan. In these, treated potato tubers (cut) were placed in the fall, in holes 2, 4, 6, 8, and 10 inches deep, ten seed pieces being planted at each depth. After covering the seed pieces with about two inches of soil, sufficient quantity of a heavy suspension of pure culture of *B. atrosepticus* in water was poured in the holes to saturate the soil, after which the holes were filled with ordinary soil. The following year no blackleg was observed on any of the plants which survived the winter. As might be expected, the seed pieces planted less than 4 inches deep were frozen and failed to germinate.

If the blackleg organism is not able to winter directly in the soil, is it capable of existing in the soil at all, and if so, is it able to penetrate healthy uninjured roots? To answer these questions a number of experiments were performed of which the following may be considered typical: Sufficient sandy loam soil (not sterilized) to fill 15 six-inch flower pots was placed in a tub. Fifty cc. of sterile standard nutrient broth and 100 cc. of water were added for each six-inch pot of soil, after which the soil was mixed thoroughly and potted. This group constituted the checks of the experiment. Next, sufficient amount of the same soil to fill 40 six-inch pots was placed in the tub and 50 cc. of a standard nutrient broth pure culture of *B. atrosepticus* and 100 cc. of water were added for each six-inch pot of soil, after which the soil was mixed thoroughly and potted. The quantity of inoculum added was sufficient to produce a saturated condition of the soil. Green Mountain tubers of a stock known to have been free from blackleg for four seasons were disinfected with mercuric chloride in the usual manner. After disinfection these were cut and planted before the cut surfaces had dried. Ten blackleg inoculated pots and five checks were placed in each of two large Wardian chambers, the bottom of which were lined with wet moss. The remainder of the pots were placed in the soil of one of the greenhouse benches. During the period of the experimentation (four months) care was taken to see that none of the plants suffered from lack of moisture, in fact, abundant moisture was given at all times. At intervals during the growing period the roots of half of the plants were injured by forcing a pot label down through the soil, simulating root injury in the field by cultivation. The results of this experiment are tabulated below:

TABLE VI.—Tubers planted in soil inoculated with B. atrosepticus.

Treatment	No. Plants	No. Blackleg Infections	
		Moist chambers	Greenhouse bench
Soil inoculated, roots injured	20	0	0
Soil inoculated, roots not injured	20	0	0
Check, roots injured	6	0	0
Check, roots not injured	9	0	0

It is noted from the above table that in no case was infection obtained even though many of the plants were kept in large moist chambers under conditions conducive to infection. Under similar conditions blackleg infection was obtained readily by inoculation of the causal organism into potato stems. The results obtained in this and similar experiments seem to indicate that infested soil is not the usual source of infection.

Ramsey[7] obtained negative results from his experiments to determine whether the blackleg organism is able to penetrate healthy roots, and as a result of his studies he concluded that unless the seed piece is infected at planting time, there is little chance that uninjured plants will contract the disease even though the causal organism is washed about the stem and root system. In addition to the soil experiments already described, the writers carried on other experiments to determine this point. The inoculation of sound roots of potato plants growing in water cultures was attempted. Tubers were allowed to sprout in wet excelsior and when the roots were approximately 3 cm. long, a piece of the tuber about 1 cubic centimeter in size containing the sprouts and roots was cut and suspended from a cork of a quart mason jar containing Shive's nutrient solution.* Two such cultures were prepared and the plants, after two months growth, were about 10 cm. in height and had about seven leaves each. Root growth was abundant. At this time one of the jars was inoculated by pouring about 5 cc. of a 4-day-old broth culture of *B. atrosepticus* into the solution, the other receiving the same quantity of sterile broth. Nine days after inoculation it was noted that the first plant had made no further growth and that the leaves presented a lighter color than the check, which had a good root system, healthy green leaves, and had begun to form tubers. A month after the addition of the broth containing the blackleg organism, the water culture potato plant was dead, but contrary to what occurs in blackleg infection under other conditions, there was no blackening of the roots or stem, either above or below the surface of the solution. It appeared that the organism was not able to penetrate the uninjured roots, but the toxic substances such as already referred to produced by the organism being absorbed by the roots resulted in the death of the plant. The check remained normal throughout the experiment. (Plate V.)

The water culture experiment confirms the results of Ramsey as to the inability of the organism to invade sound roots. The failure of the disease to develop in the experiments where soil was heavily drenched with the blackleg bacteria would indicate that the usual method of blackleg infection is not through either injured or uninjured roots.

Further evidence that blackleg is not disseminated ordinarily from hill to hill, was obtained from field experiments in which potato plants situated on slopes in fields were inoculated with virulent cultures of the blackleg organism. In every case, the inoculated plants showed typical symptoms and in many cases vigorous rotting of the stems followed presenting ample

*This solution was made up from ¼ normal stock solutions of the chemicals, and the following amounts are used for the dilute culture solution:

Potassium acid phosphate	72.0 cc.
Calcium nitrate	20.8 cc.
Magnesium sulphate	60.0 cc.
Water, to make	1000.0 cc.

Shive[20] found that this nutrient solution produced optimum growth of wheat and it was found that this was a good medium for potato growth.

septic material so that organisms must have been washed into the soil by rains and to the adjacent plants further down the incline. All of these, however, remained healthy during the remainder of the growing season.

Does B. atrosepticus persist in the soil?

As previously noted Morse[1] was of the opinion that blackleg did not persist in the soil, his conclusions having been drawn from observations made of fields planted to potatoes in successive years. Murphy[9] secured confirmatory evidence experimentally by planting halved tubers in blackleg hills after the diseased plants had been pulled up. No blackleg developed in any of these plants nor in the corresponding halves which were planted in another field. To determine whether the findings of the above investigators applied under Michigan conditions several experiments were carried on. In one of these conducted at the Upper Peninsula Experiment Station, where weather conditions are favorable to blackleg development, six diseased hills and the two healthy hills on either side were pulled up. The spaces made vacant by the removal of the first diseased hill and its neighbors were immediately planted with pieces freshly cut from an Irish Cobbler tuber recently disinfected with mercuric chloride. Similarly, the spaces made vacant by the removal of the second diseased hill and its neighbors on each side were planted with sets from an Early Ohio tuber. A week later Irish Cobbler and Early Ohio sets were planted in the spaces left by the removal of the third and fourth diseased hills, and two weeks later the remaining spaces were planted. Six weeks later observations were made and it was found that the Irish Cobbler and Early Ohio sets, which were planted immediately after the infected plants had been removed, produced plants in which typical blackleg infection occurred, while the sets planted in the adjacent hills produced plants which remained healthy. There was no evidence of blackleg in any of the other plants, indicating that *B. atrosepticus* is incapable of persisting in the soil for any considerable time or if it does so, it is only with the loss of its pathogenicity. This experiment shows that, in spite of the negative results of the cultures reported in Table VI, there is possible an occasional infection of a cut seed-piece, if it is planted in soil bearing diseased plants, immediately after their removal. The virulence of the organism in the soil decreases very rapidly, however.

Evidence that the blackleg organism may exist at least for a time in the soil surrounding diseased plants was obtained from the following experiment. Soil "A" was obtained directly from "blackleg" hills in a potato field; soil "B" from a sweet clover field adjacent to the potato field which contained the blackleg plants. Soil "B" was the same type as "A" and the sample was taken at a distance of not more than six feet from the border between the two fields. Soil "C" which was of somewhat similar sandy loam type was obtained from soil which had been inoculated artifically nine months previously with a pure culture of *B. atrosepticus,* and which after an unsuccessful attempt to secure blackleg infection by planting freshly cut seed pieces in it, had been placed in a large box and stored under a bench in the greenhouse. The sample was taken from the center of the box where moisture was still abundant. After screening these samples of soil to remove the coarse particles, small quantities (about one-half gram) of each were placed on freshly cut raw potato slices in a moist chamber, as described previously

in wintering over experiments. Two and three days after inoculation the following results were obtained:

TABLE VII.—Persistence of B. atrosepticus in sandy loam soil.

Sample	Source	No. slices inoculated	No. rotted by B. Atrosepticus	
			In 2 days	In 3 days
Soil "A"	Blackleg hills	30	2	9
Soil "B"	Sweet Clover field	30	0	0
Soil "C"	*B. atrosepticus* soil inoculation made 9 months previous	30	0	0
Check	Pure culture of *B. atrosepticus* in broth	10	10	10

Two repetitions of the above tests with soils "B" and "C" gave similar negative results. That the rotting in the case of Soil "A" was due to *B. atrosepticus* was proved by isolation of the causal organism and reinoculation of potato slices which was followed in 48 hours by characteristic symptoms.

Contrary to the findings of other investigators, it is apparent from the above experiments that the blackleg organism is present in the soil surrounding diseased plants. It does not, however, seem capable of existing there for any considerable length of time.

CAUSE OF THE DECLINE OF B. ATROSEPTICUS IN SOIL

The experiments of Giltner and Langworthy[18] on longevity of non-spore-bearing bacteria in the soil would lead one to expect that *B. atrosepticus* would be able to exist in the soil for long periods of time. It is apparent from experiments described above, however, that some other factors are involved which cause a rapid decline of *B. atrosepticus* in the soil.

Fulton[21] found that the decline of *Pseudomonas citri* in the soil is very rapid, reaching the vanishing point in about two weeks. This investigator found that the rate of decline was dependent upon several factors such as the amount of moisture present, temperature, and the deleterious effect of certain soil organisms. These factors may be concerned also with the decline of *B. atrosepticus* in the soil, but the writers wish to point out the significance of another factor which may have a very far-reaching influence, not alone in bringing about the decline of the organism in the soil but in its effect on disease production.

Following the methods described by d'Herelle[23] for the isolation of the bacteriophage, there was isolated from a sample of Soil C used in the experiments just described a lytic principle or bacteriophage in the terminology of d'Herelle. A small quantity of soil was incubated in sterile nutrient beef broth for 48 hours after which it was filtered through filter paper lined

with infusorial earth. The liquid was then passed through a Pasteur bougie (L7) and a filtrate obtained which remained free from bacterial growth and which when tested against 24-hour cultures of *B. atrosepticus* was capable of producing marked clearing of the culture.

Mallman and Hemstreet[22] have isolated from rotting cabbage what they call an "inhibitory substance" capable of producing its effects in extremely high dilutions, their tests being carried out with a fluorescent bacterial organism found on decaying cabbage.

Additional isolation work of the same type as that described above was carried out, using as a source of lytic material a carrot which has rotted in sand and using river water. Each attempt gave a filtrate capable of producing marked lysis of *B. atrosepticus* cultures. By repeated passage through cultures the lytic activity of these filtrates has been increased. A more detailed account of the work on these lytic agents as related to plant pathogens is to appear in another publication.* The accompanying plate (VI) illustrates typical results which follow adding the lytic agent to a broth culture of *B. atrosepticus*. The securing of this lytic agent from such a wide divergence of sources as well as the results of Mallman and Hemstreet would seem to indicate its wide occurrence in nature. It seems extremely likely that this lytic principle may be extremely important in reducing the number of *B. atrosepticus* individuals in soil.

This lytic principle is capable of preventing the normal pathogenesis of *B. atrosepticus* as is shown by the following experiment. Rural New Yorker tubers were disinfected with mercuric chloride, 1-1000, for 30 minutes. When dry these were cut into slices about 1 cm. thick and placed in moist chambers. In all eight slices were used, two of which were inoculated with one loop-drop of a 24-hour broth culture of *B. atrosepticus* for checks. The remaining slices were inoculated first with a loop-drop of a filtrate which contained the "inhibiting substance" and then with a loop-drop of the same broth culture as used in the checks. The lytic or inhibiting effect of the filtrate on *B. atrosepticus* when inoculated on raw tuber slices after three days incubation is shown in Plate VII. It is seen that only one of the slices upon which the filtrate was placed shows bacterial growth and this is weak as compared to the checks.

It is the opinion of the writers that the lytic principle (bacteriophage) offers an explanation for the negative results obtained from soil inoculation experiments with the blackleg organism and for its rapid decline in soils.

SUMMARY

1. The blackleg disease of potatoes in Michigan is found to be caused by a bacterial organism, *Bacillus atrosepticus* van Hall. A Fusarium is not concerned.

2. The disease is considered serious both in fields grown for certified seed as well as for table stock.

3. No commercial varieties have been found to have sufficient resistance in stems or tubers to be of practical value in disease control.

4. The blackleg organism attacks other plants besides the potato many

*Phytopathology, July, 1925.

solanaceous plants are resistant. *Nicotiana rustica* is a new host for the parasite.

5. Small quantities of inoculum when placed on tuber slices lead to "oedema" formation.

6. It is found that a pectin dissolving enzyme and a toxic substance are concerned in the production of characteristic symptoms of the disease.

7. Over-wintering of the bacteria in tubers remaining in the soil, under certain Michigan conditions, occurs. Overwintering of the bacteria directly in the soil has not been found.

8. Evidence that the causal organism is not able to penetrate healthy roots is presented.

9. The presence of the blackleg organism in the soil directly surrounding diseased plants is shown, but the organism seems to disappear quickly or to lose its pathogenicity.

10. The rapid decline of *B. atrosepticus* in the soil may be due to many factors and the writers would call attention to the important role which a lytic principle (bacteriophage) may play in this disappearance.

11. The lytic substance when placed upon potato tubers prevents the rotting normally caused by *B. atrosepticus*.

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Plate I.—Potato plant affected with blackleg. Note the inky blackening of the stem and the absence of lateral roots.

Plate II.—Tubers rotting from blackleg show creamy bacterial growth surrounded by a black border. There is also a tendency toward canal formation.

Plate III.—The inherent resistance of potato tubers, when inoculated with small amounts of inoculum, is shown by oedema formation.

Plate IV.—Effect of toxic substances on potato leaves.

Plate V.—Toxic effect of *Bacillus atrosepticus* as caused by the introduction of 5 cc. of broth culture of the organism into nutrient solution in which the uninjured roots of a potato plant are submerged.

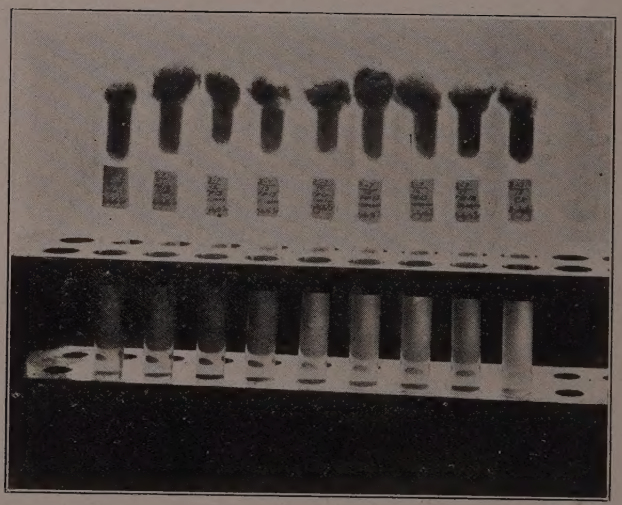

Plate VI.—Effect of various dilutions of the "lytic principle" (bacteriophage) on broth cultures of *Bacillus atrosepticus* (*B. phytophthorus*).

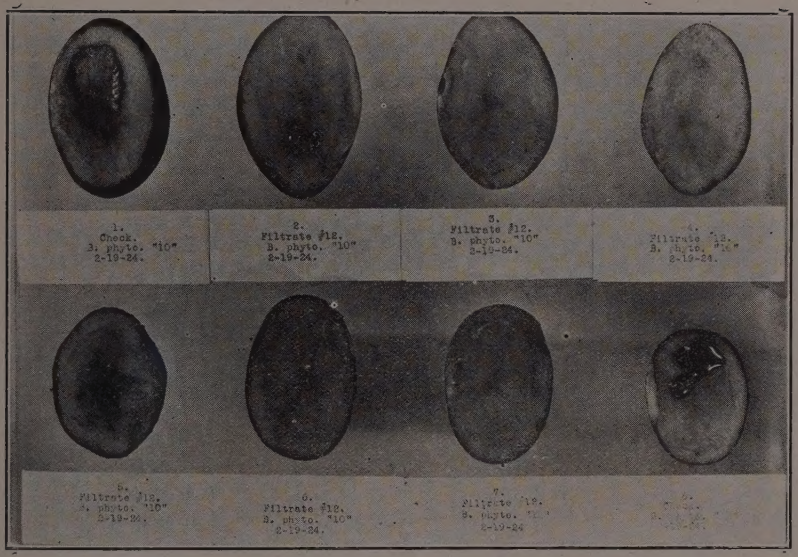

Plate VII.—The inhibitory effect of the "lytic principle" (bacteriophage) on *Bacillus atrosepticus* (*B. phytophthorus*), as shown by inoculation on raw tuber slices.